10 GAS CHROMATOGRAPHY

10.1 Introduction:

10.1.1 Gas Chromatography (GC) is a useful method for screening, separation and preliminary identification. GC provides both qualitative and quantitative information about the components of samples. Specificity is dependent on a variety of factors including stationary phase and type of detector. GC can be used to determine such things as isomers along with sufficient structurally definitive information from other techniques.

10.1.2 GC retention times of the analyte are compared to that of a known standard. The specificity of GC is increased by using two columns with stationary phases of different polarities.

10.1.3 Specific column designations, conditions and detectors utilized in casework will be denoted in the analytical case file. Positive GC results may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) along with the standard used in the comparison.

10.2 Materials:

10.2.1 Capillary Columns:

10.2.1.1 All routine methods employ gas chromatography using flexible fused silica capillary columns of 0.20 to 0.320 mm i.d.

10.2.1.2 The stationary phase is chosen to effect needed resolution. Methylsilicone (e.g., HP-1) and 5% phenylmethyl silicone (e.g., HP-5 and HP-5MS) are utilized in routine casework. The film thickness should be approximately 0.25 microns. The normal general purpose column has a 0.25 μm film thickness and 0.25 mm internal diameter.

10.2.1.3 The use of bonded, cross linked stationary phase is highly recommended, as is the avoidance of extremely thick films. If either of these qualities are compromised for special analyses, the extra column bleed generated may require more frequent maintenance of the detector. If more resolving power is required for a particular analysis, an additional, different diameter and/or phase column can be temporarily attached at the end of the existing column by using an appropriate connector. These changes should be made under the guidance of the instrument operator and must be approved by the supervisor.

10.2.2 Additional Instrument Parameters

10.2.2.1 The carrier gas is normally a high purity helium at a flow rate of 0.5 to 3 mL/min for "narrow bore" columns and 5 to 25 mL/min for "wide bore" columns.

10.2.2.2 Nitrogen makeup gas is recommended in order to support gas flow at the FID to provide optimal detector sensitivity.

10.2.2.3 Split/splitless liners designed specifically for use with the particular instrument should be used.

10.2.2.3.1 The hydrochloric acid cleaning procedure followed by solvent wash and silyl treatment is recommended. The chromic acid/ nitric acid cleaning procedure may be used if appropriate safety precautions are followed.

10.2.2.4 When the liner is to be packed, packing material should be sandwiched between layers of silanized glass wool or equivalent. If an "open tubular" liner is used, it is suggested that a small amount of silanized glass wool be inserted close to the column end. In either case, the liner will require "on column" silyl treatment when first installed.

10.2.2.4.1 Liner packing material: The solid support should be either Chromasorb W-HP or Gas Chrom Q. Mesh size should be 80/100 or 100/120 mesh. Stationary phases such as OV-1, OV-17, OV-7, and SE 30 series or their equivalent at 2-5% loading may be utilized.

10.2.2.5 The use of a "two hole" capillary ferrule allows two capillary columns of slightly different polarities to be connected into the same injection port. The sample is analyzed on two columns with a single injection of typically less than 5 μL.

10.2.2.6 Detectors most appropriate for normal drug analysis include both flame ionization detectors and mass spectrometers. Other specific detectors such as NPD and ECD may be used in circumstances requiring them. Retention time comparison may be accomplished with any detector. Quantitative analyses should use the flame ionization detector.

10.3 Methods:

10.3.1 Analysis conditions are generally set to allow for sample elution time to be greater than 3 - 5 times that of the solvent front. This allows the sample to interact sufficiently with the stationary phase.

10.3.2 The maximum allowable temperature program ramp rate for reproducible retention times is 20 degrees centigrade per minute for the Hewlett Packard 5890 GC and 30 degrees centigrade per minute for the Hewlett Packard/ Agilent 6890 GC.

10.3.3 In most instances injection is made in the split mode at a split ratio of 5 - 100:1. Splitless injections may be used when required to increase the amount of analyte delivered to the column and the detector.

10.3.4 Normal injection volume and sample size should be sufficient to provide 8 - 160 nanograms of analyte "on column" for the normal setup. This correlates to a 1 μL injection of an approximate range of solution concentrations of 0.5 – 10 mg/mL, based on a typical 60:1 split ratio.

10.3.5 Samples should be dissolved in n-hexane, CH_2Cl_2, $CHCl_3$ or MeOH for GC analysis. Depending on the nature of the samples, some samples must be cleaned up by extraction, but most may be directly dissolved in the solvent.

10.3.6 Sample concentrations should be approximately the same concentration as the GC standard and should be within the linear dynamic range of the chromatographic system and detector.

10.3.7 When using an integrator, the standards (1-2 mg/mL) should be on scale at an appropriate attenuation when 1-2 μL are injected.

10.3.8 For comparison purposes, a standard must be run using the same method conditions as the samples.

10.3.9 At a minimum, a blank consisting of the solvent(s) used to dissolve the samples, must be run on both the GC and GC/MS systems, when any of the following conditions are met:

- Before each analyst's series of sample runs whether manual or autosampler methods are utilized.
- No more than 10 samples can be run before another blank or standard/blank combination is required.
- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded or run between blank and sample.
- It is strongly suggested that a solvent blank be injected and properly documented immediately prior to a sample known to be extremely weak.
- Additional blanks may be run at the examiner's discretion.

10.3.9.1 The solvent blank must be of at least as large an injection volume of the same solvent as the sample to be injected. The upper limit injection volume is normally 4 μL.

10.3.9.2 Any significant peaks in blank chromatograms must be properly investigated and documented in the referenced case file.

10.3.10 In all instances, the GC standards file may be referred to for chromatographic conditions. Broad screening methods can be surmised from these files.

10.3.11 Integrated retention times for analytes must agree with the standard within 2 seconds (± 2 sec.) or 0.033 minutes for this to be considered a positive result.

10.3.12 Derivatization:

10.3.12.1 Some compounds, such as amphetamines or barbiturates, do not chromatograph well. Derivatives may need to be made to help effect good chromatographic peak shape.

10.3.12.2 Procedures:

10.3.12.2.1 Acetyl derivatives – appropriate for primary and secondary amines

- The acetyl derivative of phenethylamines is made by drawing up 1 μL of sample followed by 1 μL of acetic anhydride, separated by an air bubble. Acetyl derivatives generally have a longer retention time than the underivatized compound and may require a higher temperature than the underivatized compound.

- These derivatives can also be formed prior to injection by heating the sample and derivatizing reagent (~ 70° C) in a closed vial.

10.3.12.2.2 Alkyl derivatives – appropriate for barbiturates

The methyl derivative of barbiturates is made by the same procedure as listed above, only using trimethylanilinium hydroxide (TMAH) instead of acetic anhydride. Methyl derivatives often have a shorter retention time and may require a lower temperature than the underivatized compound.

10.3.12.2.3 Silyl derivatives

10.3.12.2.3.1 Silyl derivatives are often very helpful in the analysis of compounds that exhibit chromatographic difficulties due to polar functional groups such as alcohols, amines, acids, and phenols (e.g., GHB, morphine). Silyl derivatives exhibit an M-15 and M-57 peak and sometimes do not exhibit a molecular ion peak in electron impact (EI) mass spectrometry.

10.3.12.2.3.2 There are several good silylation reagents available from Regis Chemical Co., Pierce Chemical Co. and others which are designed for various applications. Catalogues from these companies are quite useful in determining the most useful derivatizing agent and their application procedure. BSTFA and BSTFA with 1% TMCS work well as silylating reagents for drug compounds.

10.3.12.2.3.3 A suitable aprotic solvent (e.g., pyridine, chloroform, toluene) may be used to dissolve the analyte and the manufacturers' directions must be followed carefully to achieve the desired result.

10.3.13 Split/Splitless Liner Cleaning and Preparation

10.3.13.1 Liner Cleaning with HCl

- Remove old column material.
- Fill with 1 N HCl and soak for at least 8 hours (overnight is fine). If the acid solution is discolored after use, replace it and continue to soak until no color change is observed.
- Wash thoroughly with distilled water.
- Wash thoroughly with methanol.
- Dry thoroughly in the oven.
- Fill with silylating reagent (10% dichlorodimethylsilane in toluene) and let stand at least 2 hours, preferably overnight.
- Wash thoroughly with toluene.
- Wash thoroughly with methanol.
- Dry thoroughly in the oven.
- Repack with suitable packing sandwiched between silanized glass wool or equivalent.
- If liner is of "open tubular" type, it is recommended that a small amount of silanized glass wool or its equivalent be inserted close to the column end of the liner.

10.3.13.2 Liner Cleaning with Chromic Acid

10.3.13.2.1 **<u>CAUTION! Components of Chromic Acid are known to be CARCINOGENIC! If this procedure must be performed, appropriate personal protection must be worn and the whole procedure must be done in a fume hood!</u>**

- Empty old column material.
- Fill with chromic acid (10 -15 g of potassium dichromate in 50 mL water per 500 mL concentrated H_2SO_4) and let stand at least 1 hour. (Overnight is fine.)
- Injector end may require physical cleaning.
- Wash thoroughly with distilled water. (Until neutral pH is obtained)
- Fill with concentrated Nitric acid and let stand at least one hour. (Overnight is fine.)
- Wash thoroughly with distilled water. (Until neutral pH is obtained)
- Wash thoroughly with absolute Methanol.
- Dry thoroughly in oven.
- Fill with silylating reagent. (10% dichlorodimethylsilane in toluene) and let stand at least 2 hours, preferably overnight. This will neutralize the active sites on the glass.
- Wash thoroughly with toluene.
- Wash thoroughly with methanol.
- Dry thoroughly in oven.
- Repack with suitable packing sandwiched between silanized glass wool or equivalent.
- If liner is of "open tubular" type, it is recommended that a small amount of silanized glass wool or its equivalent be inserted close to the column end of the liner.

10.4 Quantitation:

10.4.1 Gas Chromatography utilizing a flame ionization detector is an excellent method for quantitative analysis. The preferred method is the internal standard method.

10.4.2 The Division does not require routine quantitation of drugs. When specifically requested and required to assay a sample, this is the general procedure of a suitable GC quantitation method. Specific examples are found in the sections specific to a particular compound.

10.4.3 Validation

10.4.3.1 Run standards at a minimum of 3 different concentrations over the range of interest. The target mid-range is 1.0 mg/mL. All instrument conditions must remain constant over the range. Inject each standard at least three times.

10.4.3.2 The low standard will define the method's lower limit of quantitation. The high standard will define the upper limit of quantitation.

10.4.3.3 Average the response ratios for each standard and calculate the best fit of average response ratio (y-axis) vs. concentration (x-axis) using linear regression. The plot of the fit must appear linear. The regression coefficient (R^2) must be greater than or equal to 0.99.

10.4.3.4 Back calculate the apparent concentration of each injection using the linear regression equation. Calculate the mean and % standard deviation of the apparent concentrations for each standard. Calculate the % difference of each mean from the known concentration of the corresponding standard; each value (the accuracy) must be less than or equal to 10%. Each % standard deviation (the precision) must be less than or equal to 3%.

10.4.3.5 The data and calculations for each validation will be kept in the lab of origin and a copy will be sent to the Forensic Scientist Manager I in the Central laboratory for approval.

10.4.4 General Quantitation Procedure:

10.4.4.1 Select an appropriate internal standard which will not coelute with components of the sample. Ideally, this internal standard should elute prior to the analyte of interest. In instances where this is not practical, an internal standard with a similar elution time, though later, may be chosen.

10.4.4.2 Make up an internal standard solution of known concentration, which will be used in making all standard and sample solutions.

10.4.4.3 Make up two standard solutions of different concentrations within the acceptable linear range (e.g., 1mg/mL and 3 mg/mL) in the appropriate internal standard solution as defined in the method. One of these standards will act as the standard for the one point calibration calculation and the other will serve as a check standard (control).

10.4.4.4 Prepare your sample solution as per the method.

10.4.4.5 Run the two standards, a blank of the internal standard solution, and the sample using the appropriate GC method. Inject the standard solutions at least two times to check for reproducibility. The precision must be within that specified for the method. The injection volume should be 1-2 μl.

10.4.4.6 Using the equation listed below, calculate the % purity of both the check standard and the sample.

10.4.4.7 If the concentration of the check standard is within 10 % of the theoretical value, report the sample concentration.

10.4.4.8 If the concentration of the check standard is not within 10 % of the theoretical value, take appropriate corrective action (e.g., perform appropriate corrective instrument maintenance, the standard solutions should both be remade and repeated, the linearity of the instrument should be reevaluated prior to reporting quantitation results for a sample).

10.4.4.9 Calculations:

$$\%\ \text{Drug} = \frac{[\text{STD}] \times R_2 \times V}{R_1 \times W} \times 100$$

[STD] = concentration of standard in mg/mL

R_2 = $\frac{\text{peak area (height) of sample}}{\text{peak area (height) of internal standard}}$

R_1 = $\frac{\text{peak area (height) of standard}}{\text{peak area (height) of internal standard}}$

V = volume of internal standard solution used in mL

W = sample weight in mg

10.4.4.10 Reporting:

10.4.4.10.1 The value should be truncated to the whole integer.

10.4.4.10.2 It helps to alleviate confusion between the weight and the quantitation results when the "show form" option is utilized in FACTS.

10.4.4.10.3 Example: Heroin (Schedule I), 24.555 grams of solid material, 26 % pure.

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